of L176, and is targeted by the blocking cations, Cd^{2+} and Mn^{2+}, with single occupancy. At the intracellular side of the filter, the backbone carbonyls of T175 form the third, lower affinity site for Ca^{2+}, which mediates exit into the central cavity. This pore architecture suggests a conduction pathway involving transitions between two main states with one or two hydrated Ca^{2+} ions bound in the selectivity filter and supports a "knock-off" mechanism of ion permeation through a stepwise binding process. The multi-ion selectivity filter of our Ca_V channel model establishes a structural framework for understanding the mechanisms of ion selectivity and conductance by vertebrate Ca_V channels.

3344-Pos Board B72

Structural and Dynamic Features Underlie the Switch of Ligand Binding Specificity in a Tiam1 PDZ Domain Mutant

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The T-cell lymphoma invasion and metastasis (Tiam) family of proteins are guanine exchange factors (GEFs) for the Rho-family GTPase Rac1 crucial for cell-cell adhesion and cell migration. Deregulation of Tiam1/Rac1 signaling leads to various malignancies, including cardiovascular disease and cancer. Tiam proteins contain several protein-protein interaction domains, in particular a PDZ domain. Previously we found that the Tiam1 and Tiam2 PDZ domains had distinct binding specificities. Intriguingly, four residues in the ligand binding pocket were not conserved between the Tiam1 and Tiam2 PDZ domains. To test their importance in specificity, we engineered a quadruple mutant of the Tiam1 PDZ domain (PDZ-QM), where four residues in the Tiam1 PDZ domain were substituted for those in Tiam2. Remarkably, the Tiam1 PDZ-QM binding preference was changed to that of Tiam2. Here, we used equilibrium binding experiments and structural analyses to investigate the origins for this altered specificity. Ligand-free and -bound PDZ-QM crystal structures showed that enlarged P_0 and P_{-2} ligand binding pockets and a favorable electrostatic interaction at the P_{-4} sub-pocket were critical for the changed specificity. Biochemical studies indicated that Tiam1 PDZ-QM was less thermally stable than the WT, while NMR studies showed that a set of residues explored multiple conformations. Backbone (^{15}N) and side chain methyl (^{13}C) NMR relaxation studies confirmed the dynamic features of the ligand free Tiam1 PDZ-QM domain. In the presence of ligand, however, PDZ-QM dynamics were dampened. These studies provide novel insights into the structural and dynamic basis for Tiam1 and Tiam2 PDZ domain specificity.

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Transthyretin Induced Amyloidosis Interactions, Mechanisms and Potent Drugs Design

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Aging society faces the problem of deterioration of lifes' quality. Thus the research targeted on improvement of humans health is important and challenging task. The senile systematic amyloidosis, disease that is caused by unnaturally forming fibrils, affects about 25% of population over 80th year of life and may be lethal.

One of proteins involved in forming amyloidoic fibrils is transthyretin (TTR). TTR molecules occur in plasma and cerebral fluid [1] as a homotetramer, in healthy persons are responsible for thyroxine and retinol transport. TTRs' stability is essential to avoid TTR-based amyloidosis (a-TTR). A-TTR cascade is well known: the initial step of the whole process is dissociation of a tetramer into two dimers, then into monomers and - at the end - the monomer has to misfold. Such monomers are prone to fibril formation. A-TTR cascade may be accelerated by numerous point mutations [2].

Here we present results of our wide range MD investigations of various TTR variants - WT and medically relevant variants. We use protocol similar to these published in [3]. We try to determine the stabilizing influence of some new potent drugs - members of flavonoids family located within TTR channels., using our newly developed CHARMM [4] force field compatible ligand parameters set. We describe details of interactions between TTR molecules, ligands' stabilisation properties and early-stage amyloidosis mechanisms at an atomic level.

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[1] G.A. Hagen,et al. Endocrinol Metab, 1973, 37, 415-22.
[2] C.E. Bulawa, et al., Proc Natl Acad Sci USA, 2012, 109(24), 9629-34.
[3] K. Mikulska et al, Chem. Phys. Letters, 2011 521, 134-137.
[4] A.D. MacKerrel Jr., et al. J Phys Chem B, 1998, 102, 3586-3616.

3346-Pos Board B74

A Temperature Jump Relaxation Study of Dynamics of Thermophilic Lactate Dehydrogenase from Th. Maritima

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The temperature adaptation of proteins has been a hot subject for a long time. Such studies will help us understand he adaptation. Hyperthermophilic lactate dehydrogenase from Thermotoga maritima (tmLDH) demonstrates a strong stability at high temperature but low activity at room temperature, much lower than that at its optimum growth temperature. The t-jump studies indicate that a major reason for that is low flexibility. The bindings of cofactor, NADH and substrate inhibitor, oxamate can be treated with the same model as LDHs from other species. However, the active site loop close/open motions of the ternary complex after substrate binding are quite slow in contrast to that in mesophilic LDHs. With a double mutation (W203F,P105W), we further investigated the loop motion directly by monitoring the fluorescence of Trp on the loop. The results indicate that the slow rearrangement is indeed due to the active site loop close/open motions. Our study here demonstrates how temperature adapted proteins can be affected and provides insights into enzyme stability, flexibility and activity.

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Structural and Functional Basis of Tollip Association to the Endosomal Adaptor Protein Tom1

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Adaptor proteins are often committed to cellular processes that involve cargo internalization from the plasma membrane. Ubiquitinated cargo is internalized by endocytosis and delivered to early endosomes *via* intracellular vesicles. Cargo is then sorted to late endosomes/multivesicular bodies followed by, in most cases, degradation in the lysosomal compartments. Adaptor proteins, such as Tollip and Tom1, facilitate cargo sorting through their ubiquitin-binding domains. Tollip is localized to early endosomes, through binding to phosphatidylinositol 3-phosphate (PtdIns(3)P). Tom1 can also bind ubiquitin-conjugated cargo and is recruited to the endosomal membranes through its association with Tollip. The interaction of these two proteins is proposed to be involved in the lysosomal degradation of polyubiquitinated cargo. In this work, we demonstrate that binding of Tollip to PtdIns(3)P is negatively modulated by interaction with Tom1. Structural studies determine that the Tom1-binding domain (TBD) of Tollip is intrinsically disorded and folds upon binding to the Tom1 GAT domain, which also undergoes a conformational change upon binding. Intermolecular NOEs of the Tollip TBD-Tom1 GAT complex indicate that association is mainly driven by hydrophobic contacts with very high affinity. Ubiquitin binds to the Tom1 GAT domain at a site that does not overlap with that for the Tollip TBD, but the binding events are mutually exclusive and are likely driven by conformational changes in the GAT domain. Endosomal localization of Tom1 depends on the presence of Tollip in this compartment. Using fluorescence microscopy, we show that mutations in the binding interphase of the Tom1 GAT and Tollip TBD complex leads to a dissociation of the proteins and triggers cytosolic localization of Tom1. Consequently, we propose that association of Tom1 to Tollip helps to release Tollip from endosomal membranes, allowing Tollip to commit to endosomal ubiquitinated cargo trafficking.

3348-Pos Board B76

Modeling and Experimental Study of NHERF1 PDZ Domain Specificity

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Na/H Exchanger Regulatory Factor-1 (NHERF1) is a scaffolding protein containing 2 PDZ domains that coordinates the assembly and trafficking of transmembrane receptors and ion channels. PDZ domains bind a short sequence of amino acids at the C-terminus of their targets. Molecular dynamics (MD) simulations characterized interactions between NHERF1 PDZ1 or PDZ2 and the C-terminus of the type II sodium-dependent phosphate cotransporter (NPT2a), the parathyroid hormone receptor (PTHR), and NHERF1 ezrin-binding domain (EBD) (self-associated conformation). Modeling and earlier results determined optimal peptide length for experimental binding. NPT2a and PTHR (22 and 9 residues, respectively) peptides were used for fluorescent polarization measurements of solution-state affinities with isolated PDZ domains or intact protein. Mutations in the core-binding sequence permitted analysis of binding to a single PDZ domain in full-length NHERF1. The Kd for